

ACUTE TOXICITY OF HYDROMETALLURGICAL CHEMICALS TO FISH.

By

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HYDROMETALLURGICAL CHEMICALS  
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#### SUMMARY

Acute toxicity determinations were made on a group of nine chemicals that are presently used in hydrometallurgical operations in Ontario. The results of this study were derived from short-term, static toxicity tests and should be considered as relative toxicities only.

Of the nine compounds tested only three proved acutely toxic below the 1000 ppm. level to guppies (Lebistes reticulatus) and fathead minnows (Pimephales promelas). Kerosene Isopar E and Kerosene Solvesso 150 were the only compounds that may be described as "moderately toxic" to the fish species tested.

The significance of the toxicity data from this study can only be evaluated through a clear understanding of the conditions under which the compounds tested are normally employed, as individual dosage rates, treatment methods and initial dilution in the mixing zones of receiving waters may be quite variable.

## INTRODUCTION

In response to a request from the Industrial Wastes Branch, acute toxicity determinations were made on a group of nine chemicals that are presently used in hydrometallurgical operations in Ontario (Table 1). These compounds are generally utilized as solvents in metallurgical extractions and are commonly mixed in the process with flotation agents, collectors and other mine-mill reagents.

The results of this study are derived from short-term toxicity investigations, therefore all values should be viewed as relative toxicities only and should not be used to assess potential impact under field conditions.

## METHODS AND MATERIALS

### Experimental Procedures

Individual compounds were tested for toxicity by means of 96-hour static bioassay procedures (Sprague 1969).

Cylindrical glass jars were used for all investigations. In the preliminary range tests, jars were 22 cm high and 17 cm in diameter. All subsequent, full-scale bioassays utilized five gallon capacity containers, 27 cm in diameter and 48 cm high.

All glassware was cleaned with 95% Ethanol and rinsed thoroughly between tests to avoid cross-contamination.

Dechlorinated tapwater of approximately 140 ppm hardness ( $\text{CaCO}_3$ ) was used throughout. Test water temperatures varied from 17.0 - 21.0 C but did not vary by more than 1.0°C for any individual full-scale bioassay.

All tests were conducted without artificial aeration to minimize loss of volatile materials. During the experimental period, dissolved oxygen levels never decreased below the 50% saturation level (Table 2).

Laboratory cultured guppies (Lebistes reticulatus) were used in all preliminary range tests to locate the critical concentration ranges for each chemical. These fish were raised at 21.0° - 22.0°C, then acclimated to the test temperature at least ten days before actual testing. Fish sizes ranged from 15-21 mm, weight from 0.08 - 0.16 grams per fish. The other test organism used in the evaluations was the fathead minnow (Pimephales promelas), obtained from a hardwater pond in Vaughan Township. Minnows were acclimated to test conditions for about six weeks in 50 gallon holding tanks. Sizes ranged from 40-59 mm and weights from 0.73 - 1.89 grams each. Both fish species were starved for about 48 hours before commencement of bioassays. No mortalities were encountered in either species during the acclimation periods and all appeared to be in excellent condition.

The relation between weight of guppies and volume of test solution in the preliminary testing did not exceed 0.34 grams per liter of the test solution, while the maximum loading in full-scale bioassays using the same species was 0.03 grams per liter. The fish weight/volume relationship in bioassays employing fathead minnows was approximately 0.70 grams per liter of test solution.



Solution depth in the two liter range tests was 10.5 cm, while in the ten liter bioassays it was 20.5 cm; the former being somewhat below the 15.2 cm depth suggested for volatile material testing (Doudoroff 1951). All surface area/volume ratios were consistent in each test series but varied between the 2 liter and 10 liter tests ( $2L \approx 1:116$ ;  $10L \approx 1:57$ ).

Preliminary testing was carried out using two fish per concentration. Subsequent full-scale tests used two replicates of each test dilution, each with five fish. Selections of experimental concentrations were based on progressive bisection of intervals on a logarithmic scale (Doudoroff 1951).

A 0.1% stock solution was prepared for all preliminary and full-range bioassays. Since the chemicals were poorly soluble in water an ultrasonic probe was used to ensure a uniform dispersion of the test material (Sonifier Cell Disruptor, Model #W.185). The resulting homogeneous emulsion was used as a stock solution for preparation of the test concentrations. Constant mixing speed (output control 8) was maintained for four minutes for each Batch, with continuous cooling provided during the mixing period to minimize volatilization of aromatics. The probe was inserted just below the surface of the liquid medium to ensure sufficient agitation of the oily material.

Mortality in each test tank was recorded at 12-hour intervals and all dead fish were removed immediately. Readings of temperature, pH and dissolved oxygen were taken every 24 hours.

Fish mortality data were converted to cumulative percentage mortality for a predetermined test period. Subsequent plotting of the percentage survival data against log concentrations enabled the 50% survival value (LC50) to be calculated by the straight-line graphical method (Doudoroff 1951).

## RESULTS

Of the nine compounds tested only three proved acutely toxic below the 1000 ppm v/v level (Table 5). Kerosene Isopar E and Kerosene Solvesso 150 were the only two that could be classified as "moderately toxic" (LC 50's 1 - 100 ppm v/v) to the fish species tested. The other compound exhibiting acute toxicity below the 1000 ppm level was Kerosene NS-144, but it proved non-toxic at the next lowest concentration of 320 ppm. To establish LC50 values for the material it would have been necessary to work with concentrations in the 320 and 1000 ppm range, but at these levels a considerable layer of oil was encountered in the test solutions, which would have interfered with the interpretation of the bioassay results. Therefore it was decided not to subject Kerosene NS-144 to intensive testing.

### KEROSENE ISOPARE AND KEROSENE SOLVESSO 150

Figures 1 - 4 represent the bioassay results obtained for the two materials; comparative LC50 values are shown in Table 6.

Most of the mortality occurred within the initial 48 hours of the test periods, the exception being Kerosene Isopar E tested against the guppy, where only 60% mortality occurred within this period.

When comparing the toxicity results for both species, guppies appeared to be more resistant than fathead minnows, although the observed differences were slight and probably insignificant when considering the possible variations introduced by ununiform mixing of the test materials, as well as differences in dissolved oxygen levels.

## DISCUSSION

Although no obvious mixing difficulties were observed, the immersion depth of the mixing probe can introduce an uncontrollable variable which may affect the reproducibility of the test results. Some form of modification of the mixing probe to provide standardization should be considered for future studies with these insoluble materials. The use of a suitable solvent (acetone) was considered as an alternative to achieve a homogeneous distribution of the toxicant. However, possible interactions between chemically unknown compounds and the solvent may have masked the true toxic nature of the respective compounds, and on the basis of this possibility a solvent was not employed.

Maintenance of a pure oxygen atmosphere at the water-air interface is a useful approach in achieving uniform oxygen levels in test solutions without aeration and may well be employed whenever a high degree of accuracy is desired.

The significance of the acute toxicity data presented in this report can only be determined through a clear understanding of the conditions under which the compounds tested are normally employed. This will include a knowledge of dosage rates, treatment frequencies, residual concentrations in the discharge to receiving waters, initial dilution in the mixing zone and the composition of the local fish populations. For present purposes, an arbitrary but convenient guideline might be to restrict concentrations to one-tenth of the LC50 values in any water body inhabited by fish. It would seem unlikely that materials for which LC50 values have not been established would be used at concentrations capable of producing fish mortality. However, emphasis must be placed on the absence of information covering the sub-lethal effects of the chemicals tested and the possibility that such effects might be produced at concentrations substantially lower than acutely-toxic levels.

Table 1: LIST OF HYDROMETALLURGICAL CHEMICALS TESTED

| REFERENCE NO. | TRADE NAME                        |
|---------------|-----------------------------------|
| 1             | Kerosene Isopar E                 |
| 2             | Kerosene Livestock Spray Base Oil |
| 3             | Kerosene Napoleum 470             |
| 4             | Kerosene Mentor 29                |
| 5             | Kerosene DX 3641                  |
| 6             | Kerosene Isopar L                 |
| 7             | Kerosene Shell 140                |
| 8             | Kerosene Solvesso 150             |
| 9             | Kerosene N5-144                   |

Table 2: CHEMICAL CHARACTERISTICS OF THE EXPERIMENTAL WATER

(All values except pH, temperature, &amp; conductivity expressed as milligrams per liter)

| CHARACTERISTIC   |                      | NUMBER OF ANALYSES | MEAN | RANGE      |
|------------------|----------------------|--------------------|------|------------|
| Hardness         | (CaCO <sub>3</sub> ) | 2                  | 141  | 136 - 146  |
| Alkalinity       | (CaCO <sub>3</sub> ) | 2                  | 89   | 85 - 92    |
| Chloride         | (Cl <sup>-</sup> )   | 2                  | 32   | 31-- 33    |
| Sulphate         | (SO <sub>4</sub> )   | 2                  | 31   | 27 - 35    |
| Sodium           | (Na)                 | 2                  | 14   | 13 - 14    |
| Potassium        | (K)                  | 2                  | 1.9  | 1.7 - 2.0  |
| Free Ammonia     |                      | 2                  | .16  | .12 - .19  |
| Calcium          | (Ca)                 | 2                  | 42   | 42         |
| Magnesium        | (Mg)                 | 2                  | 9    | 7 - 10     |
| Dissolved Oxygen |                      | 393                | 8.6  | 5.1 - 9.3  |
| pH               |                      | 393                | 7.9  | 7.2 - 8.3  |
| Temperature      | (°C)                 | 393                | 19.5 | 17.0 -21.0 |
| Conductivity     | (Micromhos)          | 16                 | 311  | 290 - 354  |

Table 3: KEROSENE ISOPAR E  
FISH SURVIVAL SUMMARY

| Toxicant<br>Concentration<br>ppm | Survival<br>% | Toxicant<br>Concentration<br>ppm | Survival<br>% |
|----------------------------------|---------------|----------------------------------|---------------|
| 48 Hrs.-Minnows                  |               | 48 Hrs. - Guppies                |               |
| 18.0                             | 10            | 28.0                             | 10            |
| 10.0                             | 50            | 18.0                             | 50            |
| 7.5                              | 80            | 10.0                             | 100           |
| Control                          | 100           | 7.5                              | 100           |
|                                  |               | Control                          | 100           |
| 96 Hrs. - Minnows                |               | 96 Hrs - Guppies                 |               |
| 18.0                             | 10            | 28.0                             | 10            |
| 10.0                             | 50            | 18.0                             | 10            |
| 7.5                              | 80            | 10.0                             | 50            |
| Control                          | 100           | 7.5                              | 100           |
|                                  |               | Control                          | 100           |

Table 4: KEROSENE SOLVESSO 150  
FISH SURVIVAL SUMMARY

| TOXICANT<br>CONCENTRATION<br>ppm | SURVIVAL<br>% | TOXICANT<br>CONCENTRATION<br>ppm | SURVIVAL<br>% |
|----------------------------------|---------------|----------------------------------|---------------|
| <u>48 Hrs.-Minnows</u>           |               | <u>48 Hrs. - Guppies</u>         |               |
| 18.0                             | 0             | 18.0                             | 30            |
| 15.5                             | 10            | 15.5                             | 40            |
| 13.5                             | 0             | 13.5                             | 70            |
| 11.5                             | 40            | 11.5                             | 100           |
| 10.0                             | 80            | 10.0                             | 100           |
| Control                          | 100           | Control                          | 100           |
| <br><u>96 Hrs. - Minnows</u>     |               | <br><u>96 Hrs. - Guppies</u>     |               |
| 18.0                             | 0             | 18.0                             | 30            |
| 15.5                             | 0             | 15.5                             | 40            |
| 13.5                             | 0             | 13.5                             | 70            |
| 11.5                             | 30            | 11.5                             | 100           |
| 10.0                             | 70            | 10.0                             | 100           |
| Control                          | 100           | Control                          | 100           |

Table 5: RESULTS OF 96 HOUR STATIC BIOASSAYS  
HYDROMETALLURGICAL CHEMICALS

| TEST<br>NUMBER | DESCRIPTION OF CHEMICAL           | EFFECT          | CONCENTRATION |
|----------------|-----------------------------------|-----------------|---------------|
| 1              | KEROSENE ISOPAR E                 | LC50            | 10.0          |
| 2              | KEROSENE OIL BASE LIVESTOCK SPRAY | Non-toxic       | 1000.0        |
| 3              | KEROSENE NAPOLEUM 470             | Non-toxic       | 1000.0        |
| 4              | KEROSENE MENTOR 29                | Non-toxic       | 1000.0        |
| 5              | KEROSENE DX 3641                  | Non-toxic       | 1000.0        |
| 6              | KEROSENE ISOPAR L                 | Non-toxic       | 1000.0        |
| 7              | KEROSENE SHELL 140                | Non-toxic       | 1000.0        |
| 8              | KEROSENE SOLVESSO 150             | LC 50           | 10.7          |
|                |                                   | LC50            | 14.7          |
| 9              | KEROSENE NS-144                   | Total Mortality | 1000.0        |



Table 6: RESULTS OF 96 HOUR STATIC BIOASSAYS  
 CONDUCTED WITH KEROSENE ISOPARE & KEROSENE SOLVESSO 150

| Toxicant              | <u>48 Hour LC50</u><br>(ppm) | <u>96 Hour LC50</u><br>(ppm) |
|-----------------------|------------------------------|------------------------------|
| Kerosene Isopar E     |                              |                              |
| Minnows               | 10.0                         | 10.0                         |
| Guppies               | 18.0                         | 10.0                         |
| Kerosene Solvesso 150 |                              |                              |
| Minnows               | 11.0                         | 10.7                         |
| Guppies               | 14.7                         | 14.7                         |

## REFERENCES

- DOUDOROFF, P. 1951. B.G. Anderson, G.E. Burdick, P.S. Galtsof, W.B. Hart, R. Patrick, E.R. Strong, E.W. Surber, and W.M. Van Horn. Bioassay Method for the Evaluation of Acute Toxicity of Industrial Wastes To Fish. Sewage and Industrial Wastes Journal, Vol. 23: 1380 - 1397.
- SPRAGUE, J.B. 1969 Measurement of Pollutant Toxicity to Fish. Bioassay Methods for Acute Toxicity. Water Research. Vol. 3: 793-821.

FIG 1

KEROSENE ISOPAR E guppies

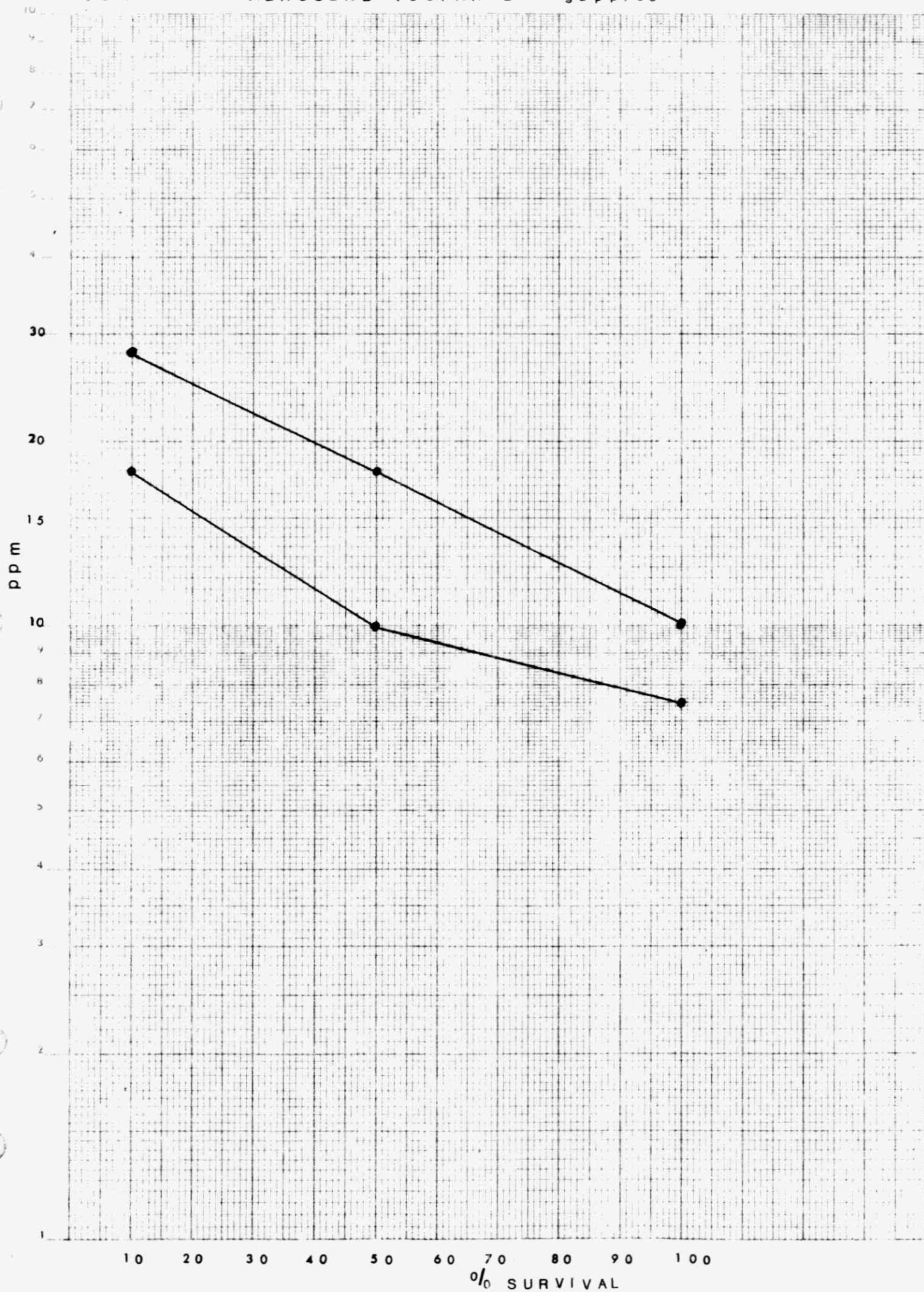


FIG 2

KEROSENE ISOPAR E

minnows

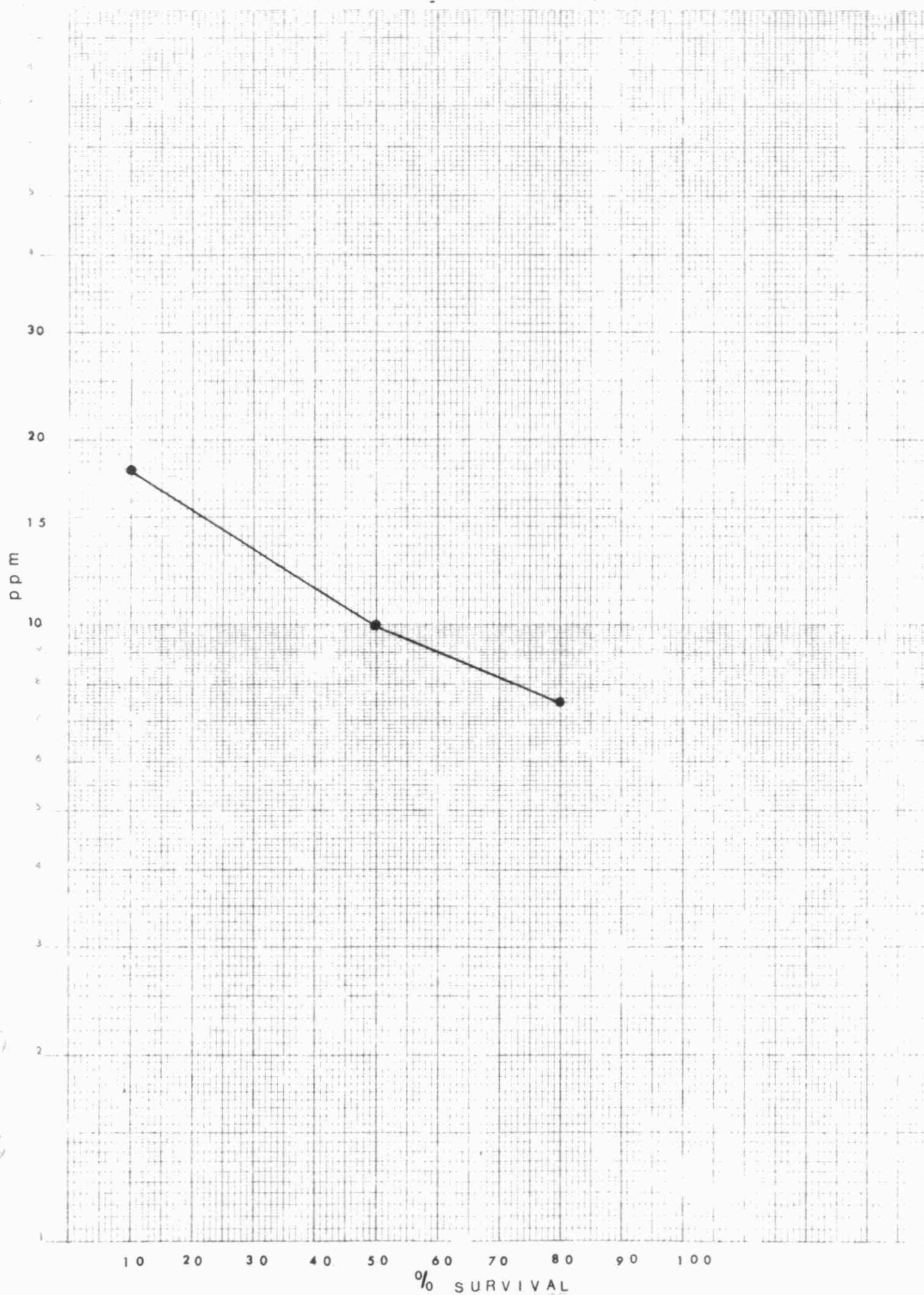


FIG 3

KEROSENE SOLVLESSO-150

guppies

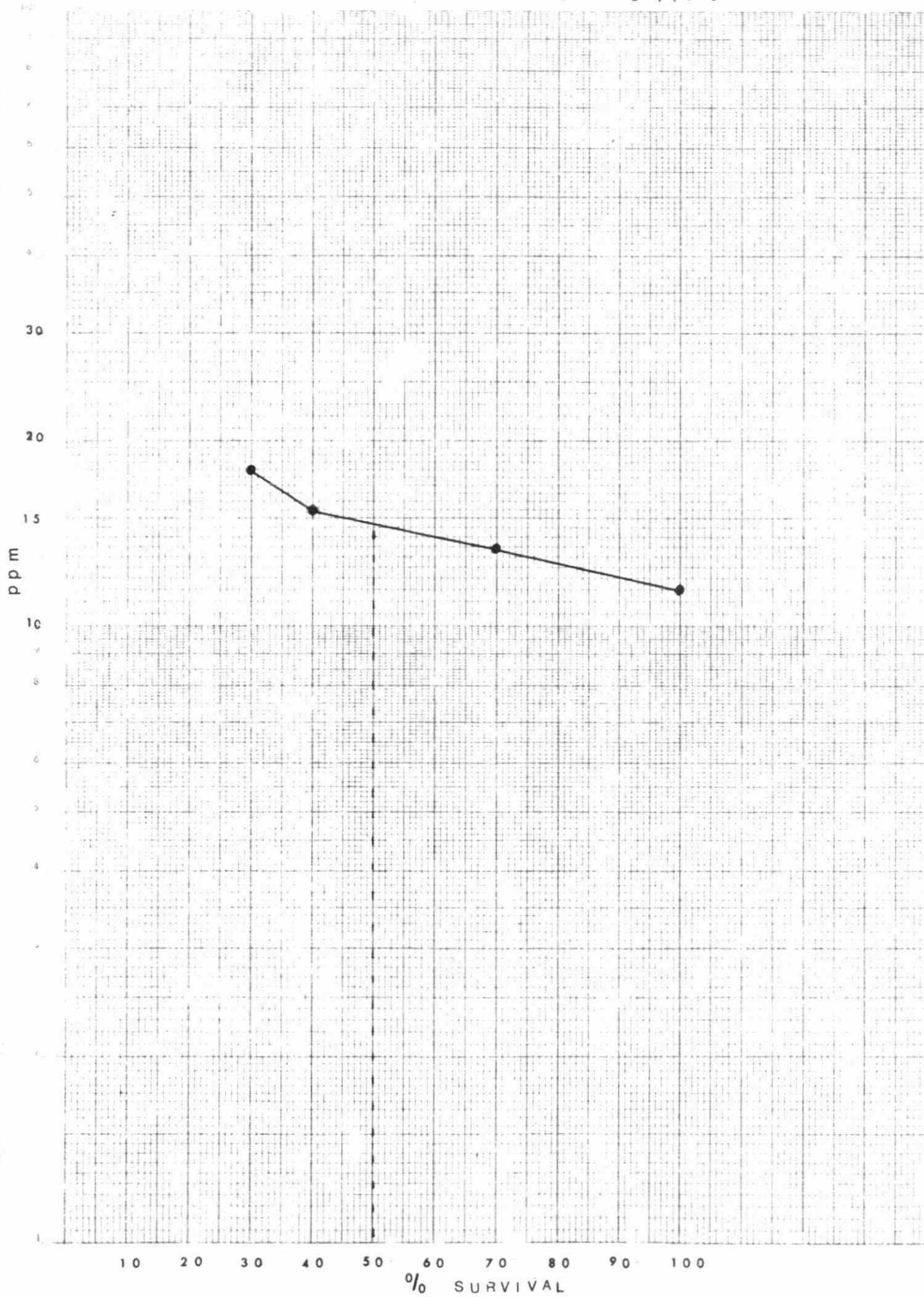
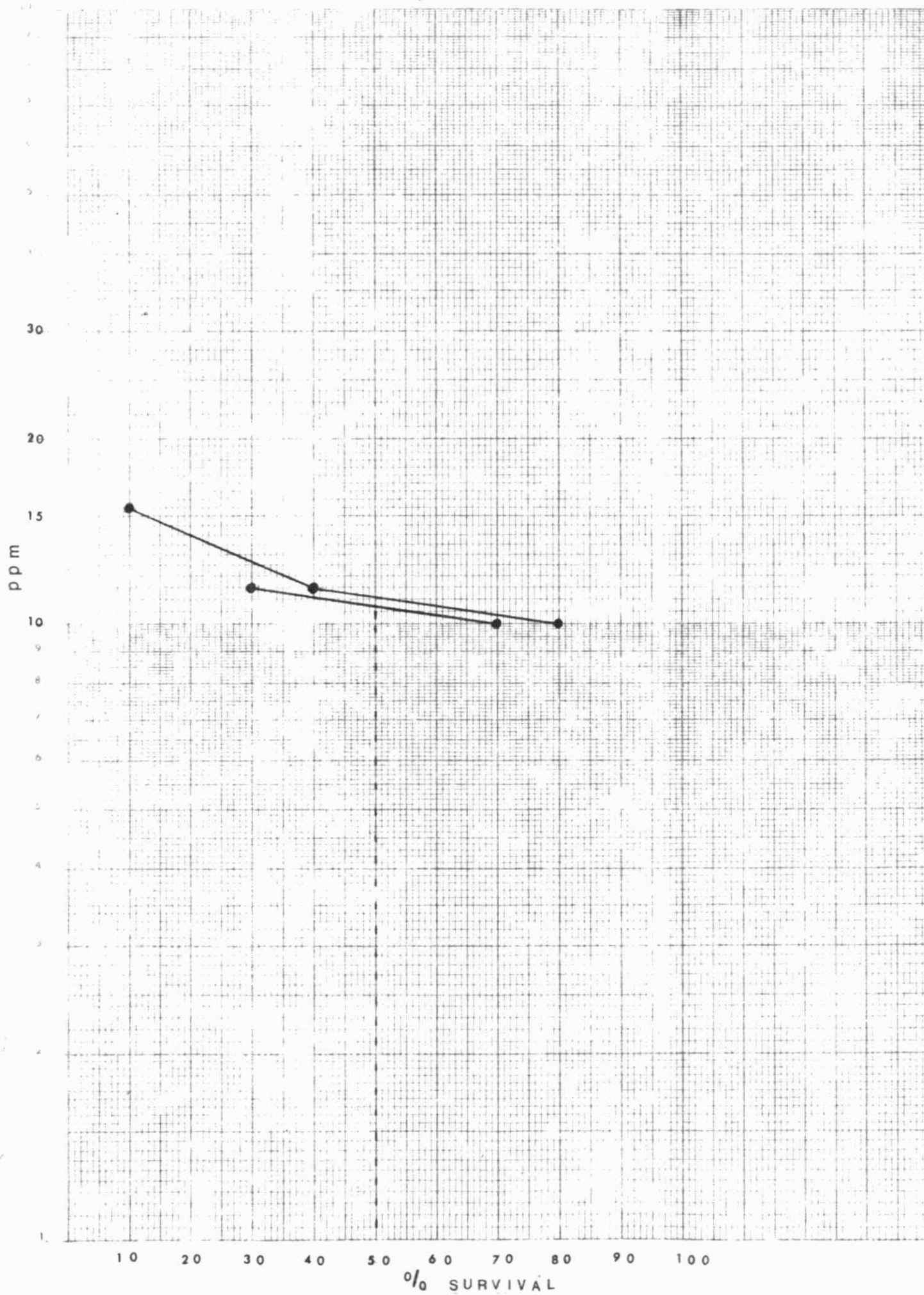


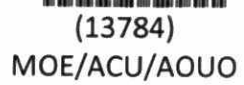


FIG 4

KEROSENE SOLVLESSO-150

minnows





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